

A QUANTITATIVE STUDY OF HEAT PRECIPITABLE  
AND ACID PRECIPITABLE PROTEINS IN THE  
HEMOLYMPH OF THE CRAYFISH, CAMBARUS BARTONI  
ROBUSTUS AS RELATED TO THE ECDYSIAL CYCLE

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## TABLE OF CONTENTS

	PAGE
INTRODUCTION AND REVIEW OF THE LITERATURE . . . . .	1
MATERIALS AND METHODS . . . . .	9
DATA AND DISCUSSION . . . . .	12
CONCLUSION . . . . .	23
LITERATURE CITED . . . . .	24

## LIST OF TABLES

TABLE	PAGE
1. <u>Orconectes</u> molt stages.	5
2. Hemolymph fractions (g/100 ml) during a normal molt cycle.	13

## LIST OF FIGURES

FIGURE	PAGE
1. Amount of heat precipitable protein in g/100 ml in the hemolymph of <u>Cambarus bartoni robustus</u> at various stages of the molt cycle.	14
2. Amount of acid precipitable protein in g/100 ml in the hemolymph of <u>Cambarus bartoni robustus</u> at various stages of the molt cycle.	15
3. Amount of total protein in g/100 ml in the hemolymph of <u>Cambarus bartoni robustus</u> at various stages of the molt cycle.	16

## INTRODUCTION AND REVIEW OF THE LITERATURE

One characteristic of all crustaceans is the presence of a rigid exoskeleton. In order for such animals to grow, they must periodically shed their skeleton and produce a larger one. The series of events referred to as the ecdysial cycle dominates the life of the individual. Time between molts becomes longer as the animal ages. Eventually in some decapods a maximum size is reached and molting stops, while others may continue to grow and molt throughout their entire life.

Structural, physiological, and behavioral adjustments result from periodic hormonal changes. Some of these changes have been described in detail (Florkin, 1960; Dennell, 1960; Maynard, 1960; Passano, 1960). However, changes in the hemolymph proteins are not well documented in the literature. Any consideration of these protein changes must be based on an understanding of the molt cycle, its stages and control.

Carlisle and Knowles (1959) and Passano (1960) have published comprehensive summaries of the endocrinology of crustacean ecdysis. The former authors have classified work in this field into three stages. The first stage began in 1928 when independent studies by Perkins, working with the prawn Palaemonetes, and by Koller, who studied the shrimp Crangon, showed that color changes of crustaceans were controlled by chemical substances circulating in the blood.

Both men found that extracts of the eyestalks when injected into experimental animals caused paling due to pigment concentration in the chromatophores. During the ensuing decade (1928-1940) most work in crustacean endocrinology was directed toward an identification of the organ in the eyestalk that was responsible for the production of this chromotropic hormone.

The removal of eyestalks from experimental organisms supported the idea that a substance was secreted by the eyestalks. When the eyestalks were ablated, a darkening of pigmentation occurred. However, an unexpected effect was also observed, precocious ecdysis. A number of investigators reported that the intermolt period was shortened (Brown & Cunningham, 1939; Abramowitz & Abramowitz, 1940; Smith, 1940; Kyer, 1942). Carlisle and Knowles (1959) mention Kleinholz's idea that the evidence of hormonal molt-control was inconclusive and concluded that pigment movements were the only function under endocrine control.

Between 1931 and 1937 Hanstrom (Carlisle & Knowles, 1959) and his colleagues studied the histology and physiology of the eyestalks, thereby describing the sinus gland and the X-organ as possible sites of hormone secretion. They investigated the effects of extracts of these two parts on the chromatophore system. A definite correlation was found between the presence of the sinus gland and the chromatophoric hormone but a relationship between the X-organ and color change was less definite.

The first period was characterized by the discovery that chromatophore activity was under hormonal control and the identification of the X-organ and sinus gland as possible sources of the chromatophoric hormones.

The second phase of crustacean endocrinology (1940-1950) was characterized by three advances: (1) a discovery that other aspects of metabolism, such as growth and development, were under hormonal control; (2) recognition that different hormones were involved in pigment movements; and (3) evidence that chromatophore-activating substances were produced in the central nervous system. Consequently, this period was dominated by a large number of experiments based on eyestalk ablation and the injection of eyestalk extracts.

Using the crayfish, Cambarus immunis, Scudamore (1942a, 1942b) supplied more evidence that eyestalk removal shortened the molt cycle and also demonstrated that other processes (gastrolith formation, oxygen consumption, calcium metabolism, and water absorption) were enhanced. Sinus gland transplants retarded these processes. His results indicated that these processes were also under hormonal control from the eyestalks.

Further evidence that the sinus gland was involved in these cyclical changes was accumulated by Pyle (1943) in his histological study of the changes in the sinus gland. He found that staining reactions changed at the time of molt and concluded that the sinus gland released substances concerned with the molt cycle.

The third period of crustacean endocrinology (1951-present) has been chiefly concerned with the relationships between the endocrine system and the central nervous system. In the first decade of this period the neurosecretory functions of parts of the central nervous system were established (Scudamore, 1947; Stephens, 1951; Bliss, 1953a; Potter, 1954; McWhinnie, 1962 & 1970). Aiken (1969) reported on the effects of light and temperature on molt. McWhinnie (1970) described seasonal influences. These studies clearly indicate a close relationship between the endocrine and central nervous systems.

A precise description of physiological changes occurring during the molt cycle and the correlation of these with the sequential events related to molt required a system of staging the molt cycle. The first universal system of staging was developed by Drach in 1939. Various parameters have since been used in staging crayfish. McWhinnie (1962) has used a gastrolith-carapace ratio as the criterion for staging Orconectes while Stevenson (1968) has employed the use of histological cuticular reactions for the same organism. A simple method of staging has been constructed by Stevenson for the crayfish Orconectes using discernable external changes in the exoskeleton. This method is as yet unpublished but the criteria are summarized in Table 1. Stevenson's system of staging has the advantage that it does not require sacrificing the animal to identify the stage.



Table 1. Orconectes Molt Stages

Stage	Characteristics
D <sub>0</sub>	The epidermis withdraws from the cuticle of the bases of the setae.
D <sub>1</sub> '	The epidermis retracts from the bases of the old setae and forms a sharp straight line a short distance below the cuticle. (Stevenson, Guckert & Cohen, 1968).
D <sub>1</sub> ''	The edge of the epidermis becomes indented and tubular new setae first become visible because of the secretion of new setal cuticle.
D <sub>1</sub> '''	Fine hairlike branches appear on each seta. The thickness and bushiness of the setae can be used to distinguish early and late D <sub>1</sub> '''.
D <sub>2</sub>	New cuticle is visible when the cuticle ensheathing a propus is pulled off. Animals must be injured to determine this stage. New cuticle is being secreted.
D <sub>3</sub>	The entire body can be compressed between the fingers. Most of the post-exuvial endocuticle has been digested and reabsorbed.
D <sub>4</sub>	The cuticle splits transversely immediately behind the carapace.
E	Ecdysis
A <sub>1</sub>	The cuticle feels slippery and very soft.
A <sub>2</sub>	The integument is no longer soft but tough and parchment-like. Stevenson (1968) proposed that the beginning of secretion of the post-exuvial endocuticle be adopted as a universal criterion to define the beginning of this stage in all crustacea.
B	This stage is not subdivided in the crayfish. The carapace begins to feel slightly brittle. The increased rigidity is especially noticeable in the post-orbital ridge and cervical groove. Although becoming rigid, this ridge and groove can still be bent readily by pressure from the fingernail.

Table 1. (Continued)

Stage	Characteristics
C <sub>1</sub>	The postorbital ridge and cervical groove become rigid. The other parts of the carapace are less flexible than before but less rigid than they will become later.
C <sub>2</sub>	The carapace is more rigid than before only the gastric region and areola are less rigid than they will become later.
C <sub>3</sub>	All parts of the carapace have achieved their final state of rigidity. Some regions especially the branchael region are still flexible, but they will become no less flexible later.
C <sub>4</sub>	Only histochemical tests of the inner cuticular layer can be used to recognize this stage. Travis (1960) reported that the membranous layer together with the epidermis can be stripped away from the rest of the integument of <u>Orconectes virilis</u> .

The molt cycle involves much more than a splitting of the old cuticle and secretion of a new one. There is also a great deal of mobilization and transport of substances needed for synthesizing the new exoskeleton (Passano, 1960). These events may be correlated with changes in the hemolymph proteins which in turn may be related to hormonal changes during the cycle.

Proteins are classified by physical properties such as solubility and heat coagulability. The properties and functions of various blood proteins are described in almost any biochemistry textbook (Kleiner & Orten, 1966; Cantarow & Schepartz, 1967). The functions of these proteins have been established for mammals; their role has been hypothetically extended to the crustacea in this study.

The heat precipitable proteins include the albumins and globulins. Albumins are involved in transport of various substances; lipids in the form of fatty acids and glycerol, glycogen, hormones, and minerals such as calcium and magnesium. They also maintain osmotic balance in the blood and serve as a source of protein nutrition for tissues. Globulins are classified into many different subclasses. They include the lipoproteins and glycoproteins. The globulins are involved in the transport of lipids, vitamins, carbohydrates, and some metallic ions such as iron, zinc, copper, and calcium. Albumins bind more calcium than do the globulins.

A classification for proteins that are not heat precipitable but are precipitated by trichloroacetic acid (TCA) has not been found.

A brief discussion of hemolymph composition during the molt cycle was made by Lockwood (1967), Florkin (1960), Maynard (1960), and Passano (1960). Concentration changes of various components have been recorded by several investigators: calcium (Travis, 1955; McWhinnie, 1962 & 1970; Crowley, 1963), sugar (Baumberger & Dill, 1928), amino acids (McWhinnie & Mohrherr, 1969; Crowley, 1963), and protein (McWhinnie, 1962; Travis, 1955; Crowley, 1963). Crowley (1963) measured an increase in 16 amino acids in a post-molt analysis of Orconectes bartoni hemolymph. McWhinnie & Mohrherr (1969) showed that  $^{14}\text{C}$ -leucine injected into the sternal sinus of molting Orconectes became incorporated into tissues in later postmolt.

In a study of the changes in blood protein composition of Panulirus argus (Travis, 1955) during the intermolt cycle, a significant rise in blood protein was observed before molt with a fall to subnormal levels at the time of molt. These subnormal levels were maintained throughout most of the post-molt period (Stages A, B, and early C). McWhinnie (1962) measured a substantial increase in blood protein in Orconectes virilis after premolt conditions were induced by eyestalk ablation. Crowley (1963) measured increased hemolymph protein in 5 species of crayfish during premolt. One protein fraction identified by electrophoresis appeared only during pre-ecdysis.

While changes in total blood protein have been studied, nothing has been done concerning changes in heat precipitable and acid precipitable protein fractions in the hemolymph. This study was undertaken to investigate quantitatively the cyclical changes of the heat and acid precipitable proteins so that a better understanding of the molt cycle might be obtained and perhaps a relationship might be established between these changes and other physiological events that are known to occur as a result of the cycle.

#### MATERIALS AND METHODS

Crayfish used in this study were collected at the Ohio State Division of Wildlife Hatcheries, Akron, Ohio, in early July and August. The animals were maintained in aerated aquaria containing 3 inches of dechlorinated tap water. Wardley's Superchlor (Wardley Products Co., Inc., Long Island City, N. Y.) was the dechlorinating agent. Dechlorinated water was periodically added to overcome evaporative losses. Aquaria temperature fluctuated between 20° and 23°C. The crayfish were fed Longlife SHRIMP-ELETES (Longlife Fish Food Products, Harrison, N. J.) and Wardley's Sturdee Shell Turtle Food. The later contained the necessary calcium supplement. They were fed every other day.

Intermolt stages were established using Stevenson's criteria for Orconectes (Table 1). All stages were used in this study except D<sub>2</sub> since the organisms had to be injured to

establish this stage. Because histological tests were not used in this study, Stages C<sub>3</sub> and C<sub>4</sub> could not be separated. Animals with fully hardened cuticles were grouped in stage C<sub>3</sub>-C<sub>4</sub>.

Animals in naturally-occurring molt stages were used whenever possible. Molt was induced by eyestalk ablation in some animals. Animals were prepared for ablation by placing them in ice for approximately 30 minutes. Eyestalks were removed at their bases with cuticle scissors. After removal the animals were returned to the ice bath for 1 hour to check blood loss and to aid recovery from surgical stress before being returned to the aquarium.

Before animals were used for experimentation, they were acclimated to laboratory conditions for several days. Hemolymph was extracted with a B-D Plastipak Tuberculin Syringe, 1.00 ml capacity. The needle was inserted into the sternal sinus through the coxal membranes of the third to fifth periopod (McWhinnie, 1962 & 1970). The blood sample was quickly transferred to a 15 x 100 mm test tube and immersed in a boiling water bath for several minutes. The heat precipitable protein portion formed a pellet in the bottom of the tube. This pellet was broken up with a glass stirring rod to release entrapped fluid and centrifuged at 3000 rev/min for 15 minutes in a Junior Angle Centrifuge having a 9 inch head diameter (Model 1600). The supernatant fluid was drawn off with a micropipette and transferred to a second test tube. The remaining pellet was broken up a second time and

resuspended in the entrapped fluids and centrifuged as before. The additional supernatant fluid was drawn off and added to the second test tube.

The heat precipitate was filtered on preweighed filter papers, Whatman #44; washed with several portions of distilled water; dehydrated by several rinsings with acetone; and heat dried overnight in an oven set at 49°C. The filtrate obtained by washing the precipitate with distilled water was treated with trichloroacetic acid (TCA) but no precipitate was formed.

The second protein portion was obtained by adding 5% trichloroacetic acid to the supernatant fluid drawn off after centrifuging the heat precipitate in the proportions of 1 part TCA to 5 parts supernatant fluid. According to McWhinnie this TCA-supernate mixture was stored in the refrigerator overnight. The mixture was centrifuged at 3000 rev/min for 15 minutes, filtered, washed with water and acetone, and dried overnight in a 49°C oven. TCA was added to the water washings of the acid precipitable protein but failed to cause any more precipitation.

After heat drying the precipitates, they were placed in preweighed bottle and stored in a  $\text{CaCl}_2$  dessicator for 4 hours before reweighing to allow an equilibrium to be reached. Knowing the weight of the precipitate, the amount of protein per 100 ml of hemolymph can be calculated.

## DATA AND DISCUSSION

The mean values of the total protein, heat precipitable protein, and acid precipitable protein fractions observed at various stages of the molt cycle are shown in Table 2. Figures 1-3 show 95% confidence limits of the data.

The heat precipitable protein portion (Fig. 1) of the hemolymph reaches its highest concentration during early Stage D ( $D_1''-D_1'''$ ). The lowest measurements occur in Stage  $D_3$  and possibly in Stage B. The latter value is of doubtful significance as it is based on only one measurement (Table 2). A slight elevation of this portion occurs in  $D_0$  over Stage  $C_3-C_4$ . However, this protein fraction does increase in Stage  $A_2$  over Stage  $D_3$ .

The acid precipitable protein fraction (Fig. 2) shows more overlapping of confidence limits than does the heat precipitable fraction. An elevation of the acid precipitable portion appears during Stages  $D_0$  and  $A_2$  over the intermolt  $C_3-C_4$  stage. A rise in this portion in Stages  $D_1''-D_1'''$  is not conclusively shown in Figure 2. However, a definite depression of the acid precipitable portion appears in Stages  $D_1'''$  and  $D_3$ . The  $D_3$  measurement is slightly elevated over the  $D_1'''$  measurement. The significance of the quantity of the acid precipitable protein measured at Stage B cannot be ascertained from the limited data available (Table 2).



Table 2. Hemolymph Fractions (g/100 ml)  
During a Normal Molt Cycle

Stage	Heat Ppt. Fraction Ave. S.D. No.	Acid Ppt. Fraction Ave. S.D. No.	Total Protein Ave. S.D. No.
D <sub>0</sub>	4.91 0.15 4	1.22 0.11 3	6.06 0.01 3
D <sub>1</sub> '	4.37 0.63 8	0.96 0.42 7	5.36 0.21 6
D <sub>1</sub> ''	5.36 0.86 5	0.89 0.54 4	5.92 0.36 4
D <sub>1</sub> '''	5.10 0.61 3	0.37 0.14 2	5.13 0.90 2
D <sub>2</sub>	-----	-----	-----
D <sub>3</sub>	3.64 0.19 5	0.59 0.23 5	4.22 0.31 5
D <sub>4</sub>	-----	-----	-----
E	-----	-----	-----
A <sub>1</sub>	-----	-----	-----
A <sub>2</sub>	4.42 0.21 3	1.26 0.14 2	5.63 0.21 2
B	3.25 ----- 1	0.77 ----- 1	4.02 ----- 1
C <sub>1</sub>	-----	-----	-----
C <sub>2</sub>	-----	-----	-----
C <sub>3</sub> -C <sub>4</sub>	4.36 0.54 18	1.02 0.37 17	5.42 0.45 13

Figure 1. Amount of Heat Precipitable Protein  
In g/100 ml. In the Hemolymph of Cambarus bartoni robustus  
At Various Stages of the Molt Cycle

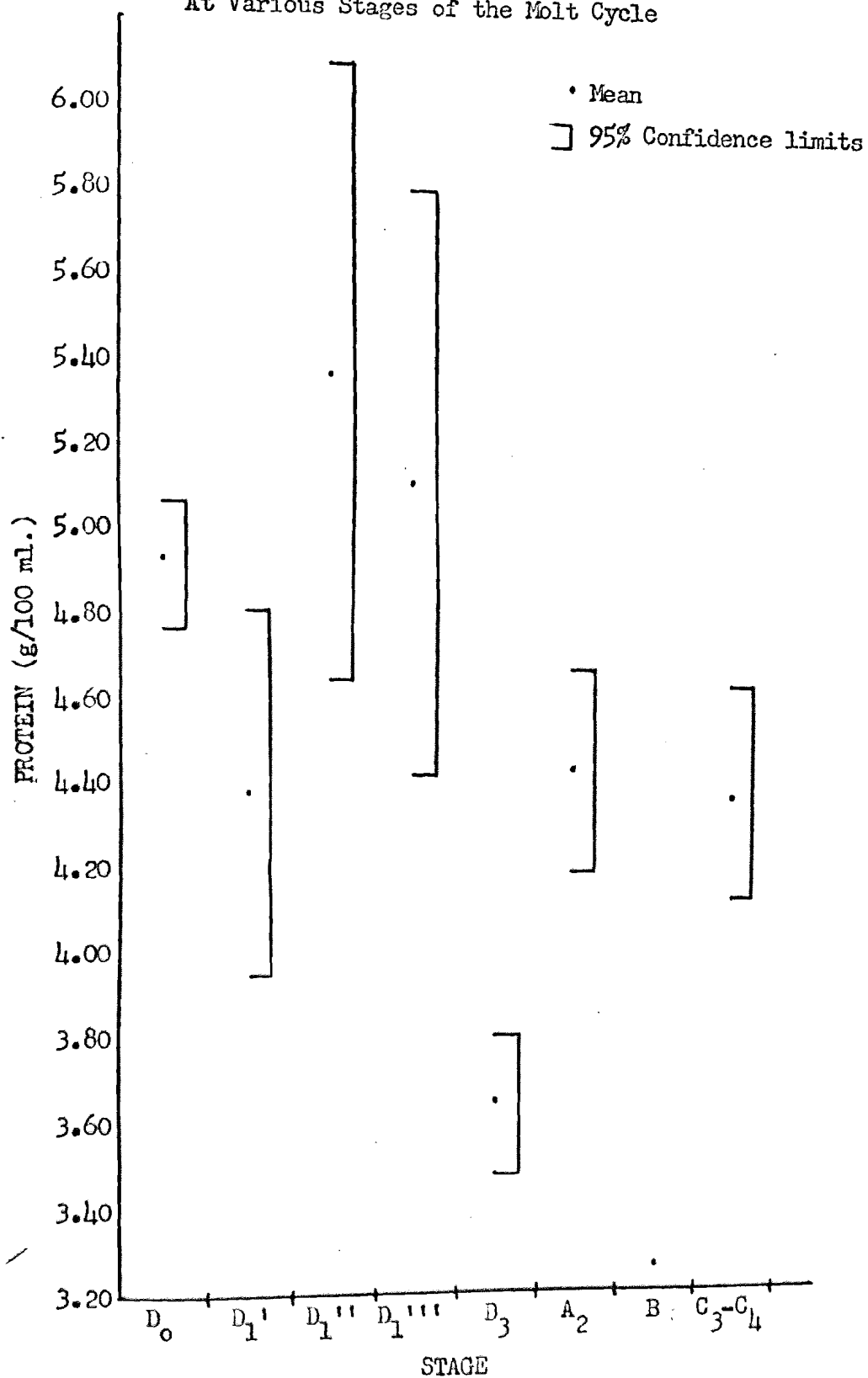


Figure 2. Amount of Acid Precipitable Protein  
In g/100 ml. In the Hemolymph of Cambarus bartoni robustus  
At Various Stages of the Molt Cycle

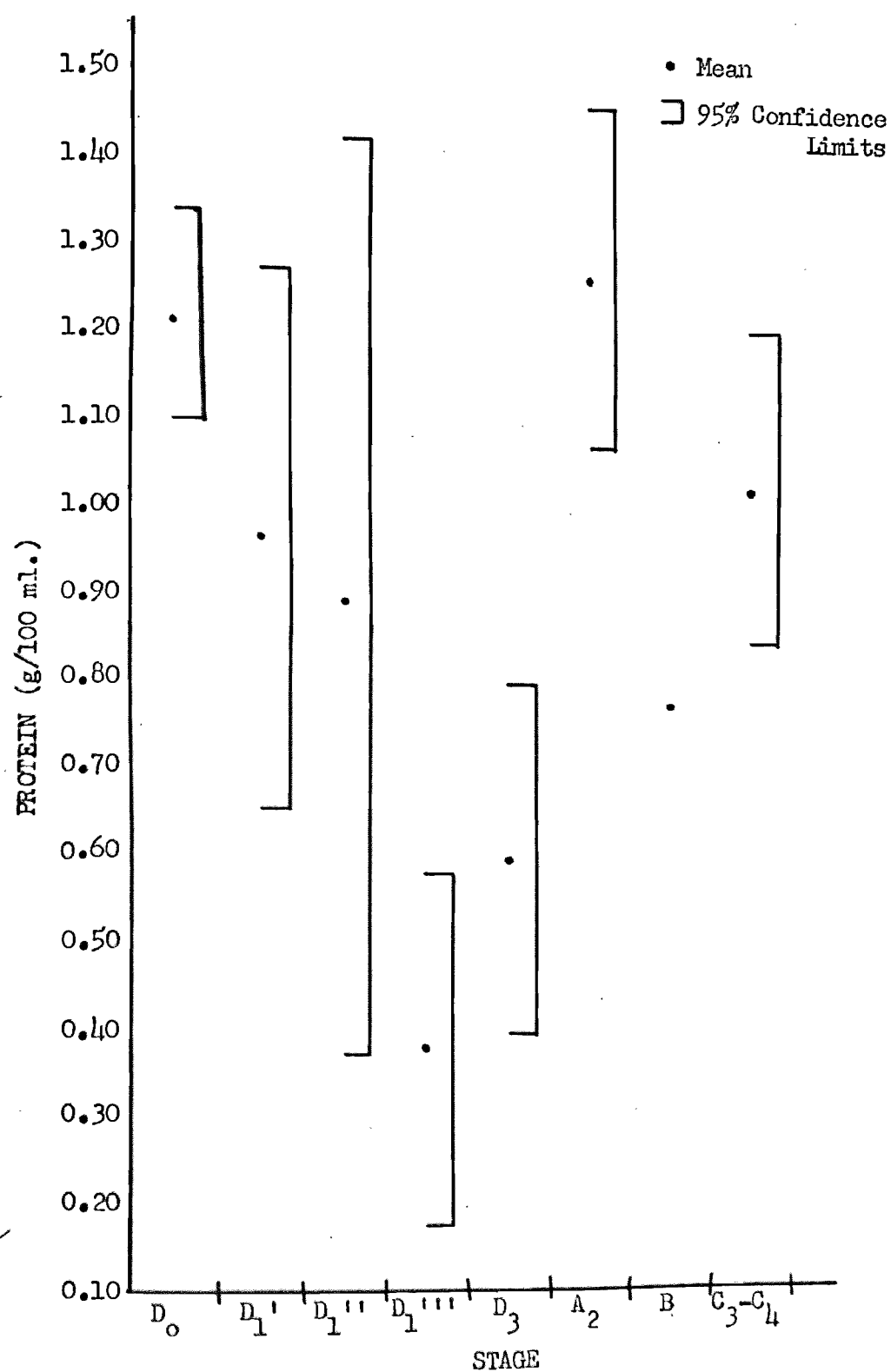
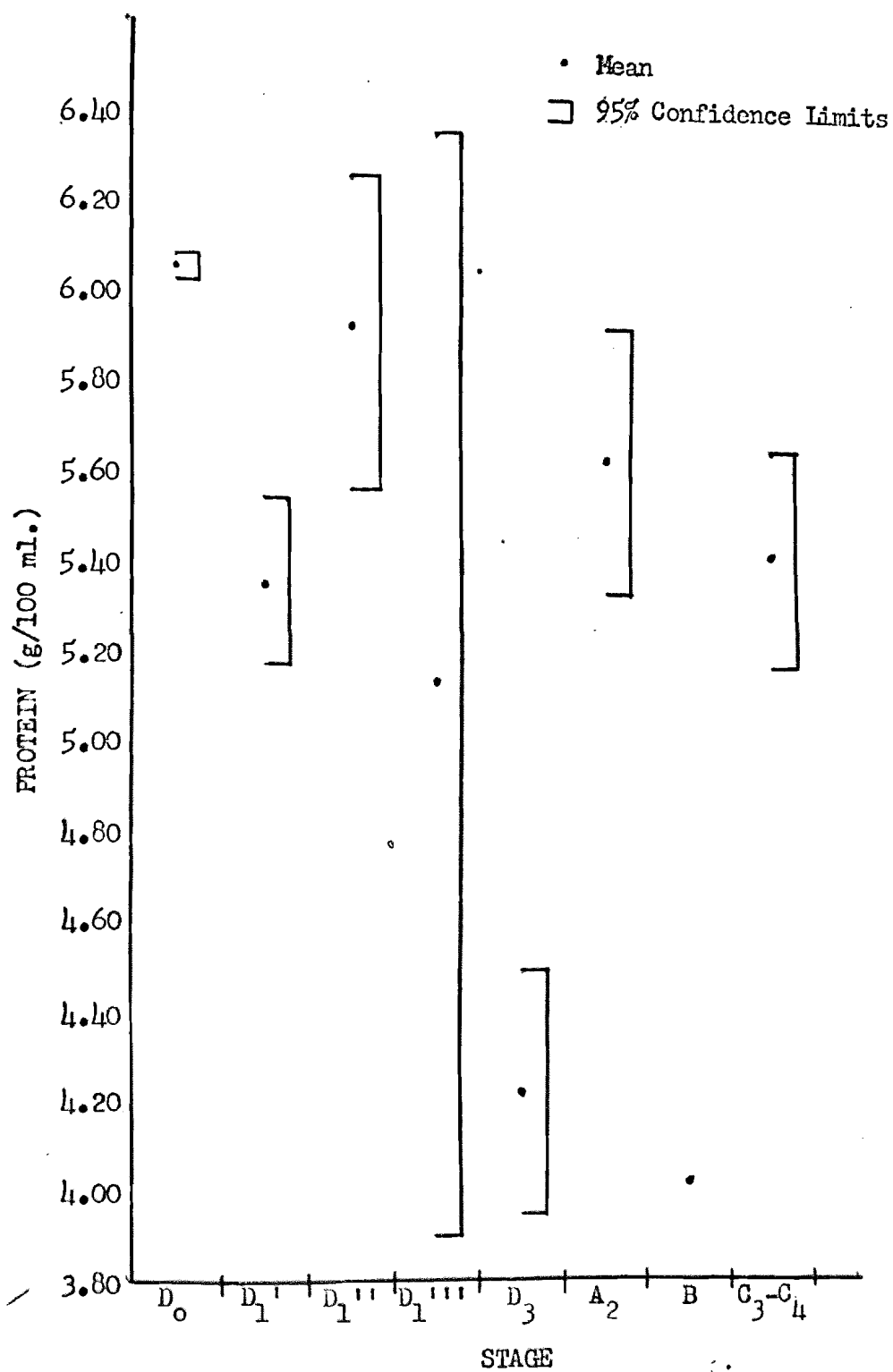


Figure 3. Amount of Total Protein In g/100 ml.  
In the Hemolymph of Cambarus bartoni robustus  
At Various Stages of the Molt Cycle



The total protein in the hemolymph (Fig. 3) shows definite elevations in concentration over the intermolt stage, C<sub>3</sub>-C<sub>4</sub>, during Stages D<sub>0</sub>, D<sub>1</sub>'', and A<sub>2</sub>. Stage D<sub>3</sub> shows a definite reduction in total protein. The confidence limits for Stage D<sub>1</sub>''' shows a wide variance making any inference from the data difficult. The total protein drops to its lowest level in Stage B. Because this is based on only one sample, its significance cannot be ascertained.

Although Stevenson's method of staging as shown in Table 1 was designed specifically for Orconectes, it proved quite applicable to Cambarus. The characteristics he used in his staging system were easily found. Waterman (ed) equates Orconectes and Cambarus in the systematic index of The Physiology of Crustacea, Part I.

In several studies eyestalk ablation resulted in premature molt (Bliss, 1953b; McWhinnie, 1962). However, ablative surgery in this study did not produce these results. Only 1 of 65 specimens successfully completed the molt. Most died within 24 hours after surgery. Abramowitz and Abramowitz (1940) observed a high mortality in Uca pugilator after eyestalk ablation. Two basic patterns seem to be apparent in crustaceans. Eyestalk removal may disturb essential functions in some decapods but not in others.

Much of the data obtained in this study corresponds to those obtained by other investigators. However, some obvious discrepancies also are apparent.

An increase in total blood protein during premolt, Stage D, has been reported in Panulirus argus by Travis (1955), in Maja by Passano (1960), and in Orconectes virilis by McWhinnie (1962). In a study of the hemolymph proteins of five species of crayfish, Crowley (1963) separated five bands of protein by electrophoresis. Four of these bands were found at all seasons. A fifth band was found only during the molting season. Passano (1960) also reported the appearance of an unknown protein fraction in D<sub>1</sub> that increased abruptly during proecdysis. Increases of amylase and tyrosinase in Astacus have also been reported (Passano, 1960). Cambarus bartoni robustus also showed increases in total protein in D<sub>0</sub>, D<sub>1</sub>'', and D<sub>1</sub>'''. The decrease of all protein measurements in D<sub>1</sub>' (Table 2, Figs. 1-3) cannot be explained at this time.

Travis (1955) and Passano (1960) have reported higher protein levels in Stage D<sub>3</sub>. A rapid decline in protein was obtained at this stage in this study (Table 2, Fig. 3). This discrepancy may be due to the increased water absorption into the blood and tissues during this stage.

In her study of Panulirus, Travis (1955) reported a decline in total protein after molt (Stages A<sub>1</sub>-A<sub>2</sub>). Although no A<sub>1</sub> animals were used in this present investigation, A<sub>2</sub> animals showed an increase in total protein as compared to D<sub>3</sub> animals (Fig. 3). This discrepancy cannot be explained at the present time.

Stage B animals showed subnormal levels of protein in Panulirus (Travis, 1955). Results of this study are consistent with hers. Stage B animals showed a depression of total protein to its lowest level.

Stage D<sub>0</sub> is characterized by a number of physiological events. Passano (1960) has summarized these. This is a period of epidermal activation with no evident histological changes in the exoskeleton. An accumulation of reserves such as calcium, lipid, glycogen, and protein occurs in the hepatopancreas. A rise in cuticular epidermal glycogen, the precursor of chitin, has been described. Gastrolith production is initiated in Orconectes (McWhinnie, 1962). Heat precipitable, acid precipitable, and total protein rises (Table 2; Figs. 1-3) over Stage C<sub>3</sub>-C<sub>4</sub>. The largest increase is in the heat precipitable portion. Since this portion probably is composed of albumin and globulins, these proteins are probably involved with the mobilization and movement of the reserve organic and inorganic materials to the sites of storage.

Stevenson (Table 1) has divided Stage D<sub>1</sub> into three periods by using morphological changes of the new setae. Physiological changes in each specific substage have not been documented in the literature. Passano (1960) listed some of the following physiological changes during the entire D<sub>1</sub> stage. A new epicuticle composed of a lipid impregnated protein is secreted by the epidermis. New setae begin to

form. Continued accumulation of organic reserves accumulated in  $D_0$  begin to decline. Passano (1960) noted that a protein fraction increases abruptly in Maja. This may be due to reabsorbed cuticular proteins from the old exoskeleton. He also reported a ten-fold rise in amylase and tyrosinase in Astacus. The tyrosinase, along with phenol-oxidase, plays a role in cuticular hardening after exuviation.

The reason for the apparent decrease of all protein measurements (Figs. 1-3) in  $D_1'$  cannot be ascertained at the present time. However, increases in later  $D_1$  ( $D_1''$ - $D_1'''$ ) of the heat precipitable portion (Fig. 1) may be due to a rise in blood enzymes responsible for the chitinization of the new exoskeleton. Calcium reabsorption is not extensive in this stage. This may account for the low acid precipitable protein fraction in  $D_1'''$  (Fig. 2).

Stage  $D_3$  is a period of extensive reabsorption of organic and inorganic materials from the old exoskeleton. Travis (1955) has reported that approximately 20% of the total organic materials and 13% of the mineral materials were absorbed. Complete absorption occurred in some regions while in most of the exoskeleton only partial reabsorption occurred. Greater absorption occurs in fresh-water forms where calcium must be conserved (Passano, 1960). He also reported abrupt increases in glucose, protein, and lipid in the blood. Hepatopaneas glycogen and lipid decreased. Lipid turnover in the hepatopaneas was due to its being converted into



glycogen and transported back to the new exoskeleton where it is converted into chitin. Increased osmotic pressure of the blood due to greater solute concentration would result in water absorption during this stage. Total protein may drop during this stage because of dilution resulting from water absorption. However, the rise in the acid precipitable protein fraction (Fig. 2) coincides with extensive glycogen and calcium mobilization. McWhinnie (1962) has suggested that the rise in blood protein in Orconectes virilis during premolt may account for the rise in total blood calcium. She found a substantial increase in protein bound calcium and a reduction in unbound calcium. Perhaps the acid precipitable portion measured in this study may be involved in the transport of the reabsorbed calcium during this stage.

Passano (1960) reported that the following events occur during Stage A<sub>2</sub>: (1) a high lipid and glycogen turnover of the hepatopancreas; (2) endocuticular deposition and mineralization; and (3) tissue growth. The increase of both the acid and the heat precipitable proteins (Figs. 1-2) coincides with these events. The increased acid precipitable protein may be involved with the transport of calcium from the hepatopancreas and gastroliths to the endocuticle. Glycogen mobilization may also have some relationship to this. Since tissue growth is also occurring during this stage, protein must be carried to the water swelled tissues to accomplish this. Albumin probably serves as a source of protein material

for the growing tissues. This may account for the slight rise in Stage A<sub>2</sub> of the heat precipitable protein. The lipid in the hepatopaneas is probably converted into glycogen.

During Stage B the secretion of the calcareous layer begins (Lockwood, 1967). Exocuticular calcification is completed after endocuticular deposition and mineralization (Passano, 1960). The fall in blood protein levels as reported by Travis (1955) has been explained by McWhinnie (1962). She states that additional calcium in an unbound form is rapidly being absorbed from the environment in Orconectes. The decreased acid precipitable protein fraction (Fig. 2) during this stage supports McWhinnie's idea. If this portion is involved with transporting bound calcium in Stage D<sub>3</sub>, a decrease in this portion would account for the increase of unbound calcium in her study. The large depression in the heat precipitable portion (Fig. 1) cannot be explained at this time.

Protein levels during Stage C<sub>3</sub>-C<sub>4</sub> (Table 2) were considered as normal values in this study since this is the period of intermolt. Tissue growth has been completed. Reserve materials obtained from food are transported to the hepatopaneas for storage until the next molt cycle. The increase of total blood protein, the acid precipitable portion, and the heat precipitable portion (Table 2) coincides with the mobilization of these materials.

## CONCLUSIONS

1. The heat precipitable protein is slightly elevated in early premolt (Stage  $D_0$ ), reaches its highest concentration in Stages  $D_1''$ , and  $D_1'''$ , and attains subnormal levels in Stages  $D_3$  and B.

2. The acid precipitable protein fraction is elevated in Stages  $D_0$  and  $A_2$ , and reaches subnormal levels in Stage  $D_3$  and B.

3. Total protein is elevated in Stages  $D_0$ ,  $D_1''$ , and  $A_2$ , and reaches subnormal levels in  $D_3$  and B.

4. Bilateral eyestalk ablation caused death in over 98% of the organisms within 24 hours after surgery.

5. Assuming that the hemolymph proteins play the same roles as they do in mammals, it may be concluded that they are involved with the transport and mobilization of materials in the crayfish.

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